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Plasma-Based Sterilization

Mounir Laroussi

Electrical & Computer Engineering Department
Old Dominion University
Norfolk, VA 23529

Rapid, safe, and effective sterilization is of the utmost importance when it comes to protecting the public in general and hospital patients in particular. Today, public health institutions face unprecedented challenges due to the advent of heat sensitive reusable medical tools and due to the appearance of heat resistant microorganisms such as prion, the protein which causes Creutzfeldt-Jacob disease, more commonly known as "mad cow disease". Conventional sterilization methods such as autoclaving and Ethylene Oxide (EtO) are inadequate in these cases. Non-equilibrium "cold" plasmas have recently been shown to be a very promising alternative, potentially capable of overcoming the above-mentioned challenges. In addition to not damaging the articles to be sterilized, cold plasmas proved to be very effective due to the synergistic effects of free radicals and UV photons, which interact with the cells of microorganisms on the atomic and molecular levels.

1. Introduction

Sterilization was concisely defined by S. S. Block [1] as "any process or procedure designed to entirely eliminate microorganisms from a material or medium". It can be achieved by chemical and/or physical means, such as heat, chemical solutions and gases, and radiation [1]. Most conventional sterilization techniques are associated with some level of damage to the material or medium supporting the microorganisms. This does not present a problem in cases where material preservation is not an issue. However, in cases where it is imperative not to damage the materials to be sterilized, conventional methods are either not suitable at all or offer very impractical and/or tedious and time consuming solutions. This situation led to a drive to develop new techniques as effective as established ones, but with added superior characteristics such as short processing times, non-toxicity, and medium preservation. Amongst these new methods, non-equilibrium atmospheric pressure plasmas have been shown to present a great promise [2]-[4].

2. Conventional Sterilization Methods

From early times human have realized that sunlight is a good sterilization agent. It offers some degree of protection from infection with pathogenic microorganisms. However, through the years, more controllable sterilization methods have been developed. Here we briefly discuss a few of these methods.

Sterilization by Heat: Both moist heat and dry heat can be used. Moist heat is generally applied by a device known as an "autoclave", which produces pressurized steam (15 psi) at 121 °C. Exposures of 20 minutes usually result in complete sterilization. Dry heat can be applied by infrared radiation or incineration. Heat sterilization is applicable only if damage by heat and/or moisture is not a problem.

Sterilization by Gases: The most widely used gas in this method is Ethylene Oxide (EtO). It is used in medical applications to sterilize heat sensitive items. Ozone (O₃) is another gas used to disinfect water and to preserve

food from spoilage. Ozone is known to interfere with cellular respiration.

Sterilization by EM Radiation: Microwaves, ultraviolet radiation, gamma-rays, and X-rays can be used to inactivate microorganisms. Each type of EM radiation affects cells in a different way. Ultraviolet radiation (UV), in the 220-280 nm range, for example, directly affects the DNA of cells. Ionizing EM radiation affects the cells of microorganisms by causing physical and biochemical changes in the DNA. EM radiation is used for the preservation of food, treatment of sewage, and for the sterilization of medical products.

Sterilization by Particle Radiation: Electrons, α -particles, and protons can be used in this method. However, electrons have been the particle of choice. In particular, electron beam irradiation is presently used for various decontamination purposes.

3. Low Temperature Plasmas

Relatively large volume low temperature plasmas are traditionally generated at reduced pressures. However, for practical sterilization/decontamination purposes, to have a vacuum system-based device would be inconvenient and expensive. Therefore, methods to generate large volume plasmas at or near atmospheric pressure were adopted. In this paper, the emphasis is on atmospheric pressure "cold" plasma devices. For an extensive coverage on the sterilization application of low-pressure plasmas, the reader is referred to reference [5].

Today, cold plasmas at atmospheric pressure can be generated by various methods. Amongst these, the Dielectric Barrier Discharge (DBD) [6], the Resistive Barrier Discharge (RBD) [7], and the Atmospheric Pressure Plasma Jet (APPJ) [8] have been especially researched in the past few years. The RBD can be driven by DC or AC power sources, the DBD requires frequencies in the kHz range, and the APPJ uses a 13.56 MHz RF power source. These devices can generate relatively large volumes of non-equilibrium, low temperature plasmas at or near atmospheric pressure.

Air or other gas mixtures can be used. The plasmas produced by these devices have typically electron densities in the $10^9 \text{ cm}^{-3} - 10^{11} \text{ cm}^{-3}$ range, plasma power densities in the $10 - 300 \text{ mW/cm}^2$, and gas temperatures generally below 100°C . They are sources of UV, visible, and IR radiation, and free radicals such as O and OH which play important roles in the destruction of microorganisms.

4. Sterilization/Decontamination by Low Temperature Plasmas

Historically, it was Siemens [9] who in 1857 used an atmospheric pressure plasma (corona discharge) to generate ozone to disinfect water. Later, Menashi [10] used a corona discharge to sterilize the surface of materials. In the mid-nineties Laroussi [2], [3] used a DBD – based diffuse discharge at atmospheric pressure to decontaminate biological media. Other experiments soon followed using various discharge configurations to destroy both gram-negative and gram-positive bacteria, as well as other microorganisms such as viruses.

Kinetics of Inactivation: The concept of inactivation or destruction of a population of microorganisms is not an absolute one. This is because it is impossible to determine if and when all microorganisms in a treated sample are destroyed [1]. Therefore, experimental investigation of the kinetics of cell inactivation is paramount in providing a reliable temporal measure of microbial destruction.

One kinetics measurement parameter, which has been used extensively by researchers studying sterilization by plasma, is what is referred to as the “D” value (Decimal value). The D-value is the time required to reduce an original concentration of microorganisms by 90%. Since survivor curves are plotted on semi-logarithmic scales, the D-value is determined as the time for a one \log_{10} reduction.

To date, the experimental work on the germicidal effects of atmospheric pressure plasmas has shown that survivor curves (CFUs versus time) take different shapes depending on the type of microorganism, the type of the medium supporting the microorganisms, and the method of exposure. Single-slope, dual-slope, and multi-slope survivor curves have all been reported. The D-value of each phase of the dual-slope and multi-slope curves are generally different (seconds to minutes range), indicating different interaction processes in these phases between the plasma and the biological media.

5. Cells Inactivation Factors

Effects of the UV: UV affects the cells of bacteria by inducing the formation of thymine dimers in the DNA. This inhibits the bacteria’s ability to replicate properly. By comparing the killing kinetics of UV radiation from a low-pressure mercury vapor lamp and that of atmospheric pressure cold plasma, Laroussi [3] concluded that UV does not play the prominent inactivation role in atmospheric pressure plasmas. This

claim was later supported by the work of Herrmann et al. [4] and others.

Effects of the Reactive Species: It has always been recognized that the reactive species, generated in a high pressure non-equilibrium discharge through electron impact excitation and dissociation, play an important role in its germicidal characteristics. Several investigators showed that discharges containing oxygen have a strong germicidal effect: The D-value decreases if a certain amount of oxygen is added. This is due to the presence, in such discharges, of oxygen-based active species such as atomic oxygen and ozone. Other radicals such as OH have also been found to play an important role in the inactivation process.

Effects of Charged Particles: Mendis et al. [11] suggested that charged particles may play a very significant role in the rupture of the outer membrane of bacterial cells. They showed that the electrostatic force caused by charge accumulation on the outer surface of the cells’ membrane could overcome the tensile strength of the membrane and cause its rupture. They claim that this scenario is more likely to occur for gram-negative bacteria, the membrane of which possesses an irregular surface.

6. Conclusions

Low temperature, atmospheric pressure plasmas have been shown to possess very effective germicidal characteristics. Their relatively simple and inexpensive designs, as well as their non-toxic nature, give them the potential to replace conventional sterilization methods in the near future. This is a most welcome technology in the healthcare arena where re-usable, heat sensitive medical tools are becoming more and more prevalent.

7. References

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